Pesticide Biochemistry and Physiology 118 (2015) 43–49

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Oxidative stress in human erythrocytes treated with bromfenvinphos and its impurities

Bożena Sosnowska [a,b,*], Bogumiła Huras [c], Bożena Bukowska [a]

[a] Department of Biophysics of Environmental Pollution, University of Lodz, Lodz, Poland
[b] Department of Hypertension, Medical University of Lodz, Lodz, Poland
[c] Institute of Industrial Organic Chemistry, Warsaw, Poland

ARTICLE INFO

Article history:
Received 4 November 2014
Accepted 21 November 2014
Available online 26 November 2014

Keywords:
Bromfenvinphos
Erythrocytes
Reactive oxygen species
Lipid peroxidation
Antioxidant enzymes
Glutathione

ABSTRACT

Bromfenvinphos (BFVF) is an organophosphorus (OP) pesticide which was widely used in agriculture and veterinary practice. During synthesis of this insecticide five main impurities are formed: dihydrobromfenvinphos, dibromo-bromfenvinphos, 2,4-dichlorophenacyl bromide, 2,4-dichlorophenacylidene bromide and 2,4-dichlorophenacylidyne bromide, which can be present in technical grade bromfenvinphos in amounts from 0.1 to 4%.

The aim of this study was to examine the influence of bromfenvinphos and its manufacturing impurities on parameters of oxidative stress, the activity of antioxidative enzymes and the level of reduced glutathione. Human erythrocytes were incubated with bromfenvinphos and its impurities in the concentrations range from 0.5 to 500 μM for 1 h.

This study indicated that 2,4-dichlorophenacyl derivatives more strongly oxidized analyzed parameters in human erythrocytes than bromfenvinphos. Investigated compounds caused an increase in lipid peroxidation and oxidation of fluorescent probe $DCFH_2$ – the strongest pro-oxidative changes were provoked by 2,4-dichlorophenacyl bromide. None of the compounds studied in the concentrations from 0.5 to 500 μM changed the activity of SOD and only 2,4-dichlorophenacyl decreased activity of CAT. The level of GSH was only altered by 2,4-dichlorophenacyl derivatives. It was observed that increasing number of bromine atoms in the side chain of those derivatives was associated with decreased GSH level.

1. Introduction

Bromfenvinphos (*E,Z*)-*O,O*-diethyl-*O*-[1-(2,4-dichlorophenyl)-2-bromovinyl] phosphate (BFVF) is organophosphorus (OP) insecticide and acaricide used in agriculture and veterinary practice. BFVF was developed by the Institute of Industrial Organic Chemistry in 1968, and then patented in 1973 [1]. In 1999 bromfenvinphos was registered as an active ingredient of Apifos – a drug against varroasis [2,3].

Varroasis is an infectious disease of the honey bees (*Apis mellifera*), which commonly occurs in almost all over the world [4]. This disease is caused by mites *Varroa destructor*, an external parasite which feed on the hemolymph of brood and adult forms of honey bees [5,6]. Bee colony attacked by *V. destructor* without treatment can live from 1 to 3 years [7]. The annual loss of bees caused by varroasis is high. For example in South America it is approximately 30%, in Europe 18–53% and in West Asia from 10% up to 85% [8]. Bromfenvinphos used as a drug for bees was highly effective (above 99%) [9,10]. Moreover, it was easy in application and did not reveal the resistance in *V. destructor* [11]. In the years 2000–2003 bromfenvinphos was the most commonly used medicine against varroasis [2]. However due to lack of established *Maximum Residue Limit* (MRL) value for BFVF in honey, this acaricide was withdrawn from use in 2003.

Taking into consideration that one of the main problems in the world is large loss of honey bee colonies as well as the deficit of the medicines against varroasis, the researchers undertook efforts to re-register bromfenvinphos as a drug for honey bees. Re-registration of bromfenvinphos requires determination of the MRL values for this compound in honey [12]. Moreover, in accordance with EU Directive 91/414/EEC [13], it is necessary to identify the impurities present in the bromfenvinphos technical concentrate and evaluate their potential toxicity. The use of acaricides is the best way to control *V. destructor*. Those chemicals may not only cause contamination of bee products, which is dangerous for insects, but can also pose a threat to human health. There are reports which have indicated that bromfenvinphos residues are present in honey bee products [2]. Consumers of honey could be exposed permanently to the bromfenvinphos. Another group exposed to this organophosphorus pesticide could be people working in the production of this

* Corresponding author. Department of Biophysics of Environmental Pollution, Pomorska 141/143, 90-236 Lodz, Poland. Fax: + 426354473.
E-mail address: bozena.szatkowska@gmail.com (B. Sosnowska).

http://dx.doi.org/10.1016/j.pestbp.2014.11.009
0048-3575/

compound or beekeepers who will use it in their apiaries. Oral route is the main exposure of humans to the pesticides from the above mentioned groups but dermal route is also important. Inhalation route occurs much less frequently, e.g. in the case of accident during manufacture of the pesticide [14].

The primary mechanism of toxic action of organophosphorus compounds (OP) is inhibition of acetylcholinesterase (AChE) activity. Many studies have also demonstrated that acute and chronic poisoning with these compounds provoked oxidative stress by enhancing the formation of free radicals and lipid peroxidation [15]. For example, it was found that malathion (*O,O*-dimethyl-*S*-1,2-bis ethoxy carbonyl ethyl phosphorodithioate), an OP compound, increased the level of lipid peroxidation [16] and altered activities of antioxidant enzymes [16,17] at lower concentrations when compared with change in AChE activity.

Previous studies on bromfenvinphos [18] showed significant changes in AChE activity in the erythrocytes even at a concentration of 0.5 μM. According to the studies mentioned above and in others [19–21] indicating the induction of oxidative stress proceeds, together with a simultaneous change in the activity of AChE by OP compounds, we wanted to find out if bromfenvinphos and its impurities affect oxidative stress in the erythrocytes.

Red blood cells (RBCs) are specific, highly specialized and the most plentiful cells in the human organism. Although their primary function is transportation of O_2 and CO_2 between lungs and tissues, these cells are equipped with effective anti-oxidative systems that make them mobile free radical scavengers, providing antioxidant protection to themselves and also to other tissues and organs in the body [22]. Erythrocytes are often used in the researches as a well-established model for the study of oxidative stress [23–26].

Moreover, oxidative damage is especially important for erythrocytes because oxidation process evoked by xenobiotics may lead to Heinz body formation and premature cell lysis which can cause anemia [27].

The aim of this study was to compare the effect of bromfenvinphos and its impurities on lipid peroxidation, ROS formation, the level of reduced glutathione (GSH) and the activity of catalase (CAT) and superoxide dismutase (SOD) of human erythrocytes. Bromfenvinphos impurities chosen for this study were: dihydro-bromfenvinphos; dibromo-bromfenvinhpos; 2,4-dichlorophenacyl bromide; 2,4-dichlorophenacylidene bromide and 2,4-dichlorophenacylidyne bromide. They can be present in technical grade bromfenvinphos in maximal amounts: 2%; 4%; 0.1%; 0.1%; 0.1%, respectively (Fig. 1). We selected a wide range of bromfenvinphos concentrations (0.5–250 μM) in this study; from very low, which changed AChE activity in the erythrocyte, what was previously recorded [28], to much higher which are likely to enter the body as a result of accidental or self-poisoning. Our research was performed after 1 h incubation, because as shown earlier [18,28,29], such incubation time was sufficient for the compounds to enter the cells.

2. Materials and methods

2.1. Chemicals

Bromfenvinphos (purity 99.9%), dihydro-bromfenvinphos (purity 99.5%), dibromo-bromfenvinphos (purity 99.6%), 2,4-dichlorophenacyl bromide (purity 98.6%), 2,4-dichlorophenacylidene bromide (purity 98.2%), 2,4-dichlorophenacylidyne bromide (purity 98.9%) (Fig. 1) were synthesized in the Institute of Industrial Organic Chemistry, Warsaw, Poland.

2.2. Erythrocytes isolation and treatments

Human erythrocytes were obtained from whole blood taken from healthy donors in the Blood Bank of Łódź, Poland. The erythrocytes were centrifuged (3000 rpm) and washed twice with phosphate-buffered saline (150 mM NaCl, 1.9 mM NaH_2PO_4, and 8.1 mM Na_2HPO_4, pH 7.4). The bromfenvinphos and its impurities, dissolved in DMSO were added to the erythrocytes to obtain final concentrations of 0.5; 5; 50; 100; 250 and 500 μM. The erythrocytes, which were incubated only with DMSO, were used as control. The final concentration of DMSO in all samples studied was 0.125% and it was not toxic to the cells [30]. The erythrocytes of 5% hematocrit were incubated with investigated compounds for 1 h at 37 °C. After the treatment, the following parameters were analyzed. Concentration of hemoglobin was performed by the method described by Drabkin [31].

2.3. Analytical methods

To measure the production of reactive oxygen species (ROS) fluorescence probe 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) was used. The rate of $DCFH_2$-DA oxidation was measured by flow cytometry. The 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate was added to control erythrocytes and the erythrocytes incubated with investigated compounds. It diffuses across the cell membrane and is hydrolyzed by intracellular esterases to 2′,7′-dichlorodihydrofluorescein ($DCFH_2$-DA), which, upon oxidation, yields highly fluorescent 2′,7′-dichlorofluorescein (DCF). The final concentration of 2′,7′-dichlorodihydrofluorescein diacetate in the erythrocytes

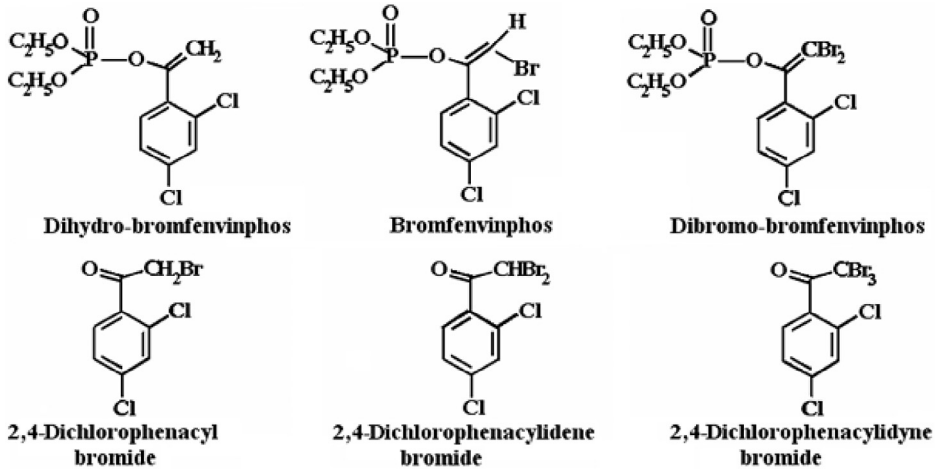

Fig. 1. Structural formulas of bromfenvinphos (BVFV) and its five impurities.